Kudoa septempunctata (Myxozoa: Multivalvulida) from the trunk muscle of cultured olive flounder (Paralichthys olivaceus) causing food poisoning of human

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INTRODUCTION
Recently, cases of a new food-borne disease of human associated with ingestion of olive flounder Paralichthys olivaceus have increased in Japan. The patients showed diarrhea and vomiting within 2-20 hours after consumption of raw fish (sashimi), however the prognosis was usually good. Epidemiological analysis and toxicity tests with experimental animals indicated that the causative agent was Kudoa septempunctata infecting the trunk muscle of olive flounder (Kawai et al., submitted). In the present study, prevalence and intensity of infection with K. septempunctata of cultured olive flounder were investigated by microscopic observation and PCR assay. We aimed to develop a practical control strategy for preventing the food poisoning of consumers.

MATERIALS & METHODS
To determine the prevalence and intensity of infection with K. septempunctata, ten fish (ca. 30-35 cm in length) were randomly sampled each time from an infected stock of cultured olive flounder in February, April and May 2011. Pieces of tissue were collected from the opercular muscle using a cotton swab, and presence or absence of spores was quickly checked by wet mount observation (St-Hilaire et al., 1997). Tissues (0.5 g) of the skeletal muscle were collected and passed through mesh screens. After spore suspensions were centrifuged, pellets were re-suspended in PBS, and number of spores were counted in a hemacytometer. DNA was extracted from the skeletal muscle tissue, and PCR assays based on the 28S rDNA were conducted according to the protocols of Grabner et al. (in preparation).

RESULTS
Gross observation
Infection with K. septempunctata in fish was subclinical, showing neither cyst formation nor myoliquefaction (Fig. 1).

Light microscopy
Spores of K. septempunctata with 5-7 polar capsules (Figs. 2-4) were clearly detected from the opercular and skeletal muscle at a same rate (Table 1).

Prevalence and intensity
Prevalence of infection was 40-50% by light microscopy but 100% by PCR. Intensity of infection (number of spores/g-tissue) varied among individual fish, between 10^4-10^6 spores/g (Table 1).

DISCUSSION
Subclinical infection with K. septempunctata suggests that sashimi containing spores would be eaten without being noticed by consumers. Intensity of infection reached a maximum of 10^6 spores/g, which is high enough to cause food poisoning in human, though lightly infected fish (PCR-positive and microscopy-negative fish) may not be a health problem. Prevention and treatment of myxozoan diseases are generally difficult, and thus it is important to detect and remove infected fish as early as possible from the stock. In the present study, detection of spores from the muscle under the operculum was found to be a simple and practical diagnostic test in fish farms. Early diagnosis of juveniles using the PCR assay and a large-scale screening of commercial-sized fish using the opercular muscle test are recommended to eliminate infected fish prior to their sale on the market. Further studies are required on the quantitative risk analysis (threshold of infection intensity causing food poisoning) to provide a basis for decision-making of selling commercial-sized fish on the market.

REFERENCE